Project No.
TITLE: Exhibit B Book No.

From Page No. 35

DATE:

TITLE: Filter Lift Development; Capture Lift

EXPERIMENT #: 092

BACKGROUND/PURPOSE: Try to reduce background binding through the use of "capture lifts". These filters are pre-treated with goat anti-human kappa and blocked with BSA to more closely mimic the ELISA format which has been working so well.

DESIGN:
1. Dilute 0.4 ml of goat anti-human kappa (Southern Biotech; 1 mg/ml) in 10 ml of PBS. Aliquot 2 X 5 ml of this stock to each of 2 X 100-mm plastic dishes.

2. Invert 2 nitrocellulose filters on the antibody solution and incubate 60 min/RT.

3. Transfer the filters to 1% BSA/PBS/azide and incubate ON/4°C.

4. Remove the filters from block, rinse 1X with TBS, and air-dry. While the filters are still slightly moist, invert on 240H2 and IX64 plates. Leave the filters on the plates 9 hrs/RT, rinse in TBS, and air-dry.

5. Set-up 4 sets of membrane incubations, as outlined below:

	5X dil. control*	50X dil. control	5X dil. biotin	50X dil. biotin
IX64 plate	1	2	3	4
240H2 plate	5	6	7	8

*1 ml membranes + 4 ml 1% BSA/PBS/azide. The 50X dilution was merely a 10-fold further dilution.

6. Test both filters for Ponceau S staining and HRP-LeY reactivity:

	IX64	240H2
Ponceau S	9	11
HRP-LeY	10	12

7. Incubate filters with membranes 4 hrs/4°C. Rinse filters with PBS/0.1% Tween 20.

8. Incubate with 1:1000 dilution of streptavidin alkaline phosphatase in 1% BSA/PBS/azide for 30 min/RT.

9. The filters were developed 15 min/RT.

RESULTS:
1. The Ponceau S staining of the filters was uniform and intense, indicating effective blocking by the BSA solution.

2. The HRP-LeY reactivity was overwhelming...the film was over-exposed within 1 min exposure. The background was negligible.

3. This approach eliminated the background staining of both IX64 and 240H2 filter-lifts with control membranes.

4. There is a hint of signal above noise when comparing the 240H2 lift with the IX64 lift.

NEXT:
1. Keep TX-100 elevated during incubation steps?

2. Remove divalent cations?

3. Keep high [ionic] to reduce hydrophobic interactions between membranes and filter (i.e. drive hydrophobic interactions prior to adding membranes to filters)?

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Witnessed & Understood by me, Date / / Invented by Date

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CERTIFICATION OF FACSIMILE TRANSMISSION

I hereby certify that this paper is being facsimile transmitted to the Patent and Trademark Office on the date shown below.

Type or print name of person signing certification: JANET Fowler

Signature: Janet Fowler Date: 2/10/06

PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

First Applicant: Jeffry D. Watkins, et al.
Serial No.: 09/977,797
Application Date: October 15, 2001
For: Methods For Identifying Ligand Specific Binding Molecules
Docket No.: X-16763B

Group Art Unit: 1639
Examiner: Wessendorf, T.
Conf No.: 3451

DECLARATION UNDER 37 C.F.R. 1.131

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

We, Jeffry D. Watkins, William D. Huse, and Herren Wu declare that:

1. We are the applicants and co-inventors of the subject matter encompassed by the claims of the above referenced patent application.

2. All the actions, experiments, events, and observations described in this declaration occurred in the United States by one or more of us, or on our behalf and under the direction and control of one or more of us as coinventors prior to July 2nd, 1997.

3. The attached exhibits A and B describe all of the steps encompassed by Claim 1 currently pending in the above referenced application. Specifically, Claim 1 recites "A method comprising:
   a) providing
      (i) a solid support coated with an anti-immunoglobulin reagent, wherein said solid support comprises a membrane and
      (ii) a phage expressed antibody library,

(b) contacting said solid support and said phage expressed antibody library to generate an antibody bound solid support;
(c) contacting said antibody bound solid support with a sample containing one or more antigen/s, wherein said contacting generates a solid support containing antibody-antigen complexes;
(d) identifying one or more antibody-antigen complexes."

4. We have reviewed Exhibit A submitted herewith and declare that Exhibit A consists of consecutive pages dated prior to July 2nd, 1997 from the notebook of co-inventor, Jeffry D. Watkins.

5. We have reviewed Exhibit B submitted herewith and declare that Exhibit B consists of consecutive pages dated prior to July 2nd, 1997 from the notebook of co-inventor, Jeffry D. Watkins.

6. Exhibit A describes an experiment in which a phage expressed library of human Fabs against lymph node antigens was screened against a cell membrane preparation made from cells expressing tumor antigens. Briefly, a nitrocellulose membrane coated with anti-human κ antibodies was used to capture Fabs from a phage expressed human lymph node library. The anti-human κ antibodies recognize and bind to the κ light chain portion of the Fabs, thereby immobilizing the Fabs on the nitrocellulose membrane. The nitrocellulose membrane with the bound Fabs was then used to screen the cell membrane sample made from cells expressing tumor antigens. To aid in detection of antigens bound to Fabs from the phage expressed lymph node library, the tumor antigens from the cell membrane were biotinylated prior to incubation with the Fab library. After washing of the nitrocellulose membrane to remove any unbound antigens, streptavidin conjugated alkaline phosphatase was added. The streptavidin conjugated alkaline phosphatase identified bound biotinylated antigens which in turn had been bound by Fabs from the lymph node phage expressed library.

7. The "capture lift" described in step 1 of Exhibit A is a nitrocellulose membrane coated with goat anti-human κ antibodies and is prepared according to steps 1-3 of Exhibit B.

8. Specifically, steps 1-5 of Exhibit A describe a nitrocellulose membrane coated with goat anti-human κ antibodies which is incubated together with a sample of a lymph node phage library expressing human IgG1/κ Fabs against tumor antigens. To serve as a positive control for detecting tumor antigens in the screening of the cell membrane preparation, a sample of the phage expressed lymph node library was mixed with phage expressing BR96-reactive Fabs. The BR96 reactive Fabs recognize the LeY-related carbohydrate, an

antigen found on many tumors cells. Step 6 describes incubating the nitrocellulose membrane from the previous steps (steps 1-5) with a sample containing membranes and membrane proteins made from cells expressing tumor antigens. In addition, this membrane preparation has been biotinylated to facilitate detection of bound antigen-antibody complexes. After an incubation of 5.5 hrs at 4°C, the nitrocellulose membrane is washed and biotinylated proteins, which have been captured by the Fabs from the phage expressed library, are detected by adding strepavidin conjugated alkaline phosphatase.

We further declare that all statements made herein of our own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. 1001), and may jeopardize the validity of the application or any patent issuing thereon.

2/9/06
Date

Jeffry D. Watkins

2/9/06
Date

William D. Hose

2/8/06
Date

Heiren Wu